15. (New claim) The fragment of claim 9 wherein the reporter molecule is a chelated metal, fluorescent compound, or a compound detected by nuclear magnetic resonance spectroscopy or electron spin resonance spectroscopy.

16. (New claim) The fragment of claim 12 wherein the fragment binds a soluble antigen.

17. (New claim) The fragment of claim 19 wherein the soluble antigen is an interleukin, viral antigen, immunoglobulin, interferon, tumor necrosis factor-α, tumor necrosis factor-β, a colony stimulating factor, or a platelet derived growth factor, or a receptor thereof.

18. (New claim) The fragment of claim 12 wherein the polymer has an average molecular weight range from 25,000 Da to 40,000 Da.

19. (New claim) The fragment of claim 12 wherein a variable region domain of the fragment comprises at least one complementary determining region from one antibody and the remainder of the variable region domain from a second antibody.

REMARKS

Claims 5 and 9-19 are pending in the present application. Claim 12 has been amended to correct typographical errors and claim 9 has been amended to separate reporter molecules from effector molecules (see new claim 13). New claims 13-19 have been added, support for which can be found, for example, at page 4, lines 7-23, page 5, lines 6-15, page 6, line 34 to page 7, line 1, and page 7, line 33 to page 8, line 4 of the published PCT specification. No new matter has been added.

I. The Claimed Invention Is Novel

Claims 5, 11 and 12 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Zapata *et al.*, *FASEB J.*, **1995**, *A1479*, Abstract 1288 (hereinafter, the "Zapata I reference").

Applicants traverse the rejection and respectfully request reconsideration because the Zapata I reference does not teach every feature recited in claims 5, 11 and 12.

The Zapata I reference reports a humanized anti-CD18 Fab' fragment containing a single free thiol in the hinge region to which 5 kDa and 10 kDa monomethoxypoly(ethylene glycol) chains are attached. The Zapata I reference does not teach or suggest that the Fab' fragment has a hinge domain that comprises not more than one cysteine residue.

The Office Action erroneously concludes that the Zapata I reference teaches a Fab' fragment "which contains a single cysteine in the hinge region..." (see, page 4 of the Office Action). The Office Action, however, does not refer to any portion of the Zapata I reference to support such a conclusion. Contrary to the assertions in the Office Action, the Zapata I reference teaches that the Fab' fragment "contains a single free thiol." The Zapata I reference **does not** teach that the cysteine residue that forms a portion of the single free thiol is the only cysteine residue in the hinge region. Indeed, many immunoglobulins have a plurality of cysteine residues in the hinge region. Applicants enclose herewith a copy of Sandlie *et al., Antibody Engineering*, Borrebaeck (Ed.), **1992**, W.H. Freeman and Company, New York, p.71, which shows a comparison of hinge region sequences of human and mouse immunoglobulins. In particular, the immunoglobulins recited in Table 3-1 each have multiple cysteine residues in the hinge region. Further, the Bodmer reference cited by the Examiner and discussed below in more detail, also teaches that the hinge region comprises between two and eleven inter-heavy chain disulfide bonds (*i.e.*, between 2 and 11 cysteine residues) (see, for example, page 2, lines 5-9 of the Bodmer reference). Thus, merely because the Zapata I reference reports that the antibody recited therein contains a single free thiol **does not** mean that the cysteine residue that forms a portion of the single free thiol is the only cysteine residue in the hinge region.

Thus, the Zapata I reference does not teach every feature recited in claims 5, 11 and 12. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102(b) over the Zapata I reference be withdrawn.

II. The Claimed Invention Is Not Obvious

A. The Combination of the Zapata I and Zapata II References

Claims 5 and 9-12 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over the Zapata I reference in view of U.S. Patent No. 6,214,984 (hereinafter, the "Zapata II reference"). The Office Action mistakenly asserts that it would have been *prima facie* obvious for one skilled in the art to have used the antigen binding fragment with polyethyleneglycol (PEG) as reported in the Zapata I reference and label the fragment and produce compositions comprising a carrier and the antibody as taught in the Zapata II reference. Applicants traverse the rejection and respectfully request reconsideration because the Zapata II reference does not cure the deficiencies of the Zapata I reference.

As a preliminary matter, the Office Action asserts that the Zapata II reference has a continuation date of April 25, 1995 (see, page 4 of the Office Action). The Zapata II reference, however, is not a continuation, via an intermediate divisional application, of application No. 08/425,763 filed April 25, 1995 (now U.S. Patent No. 5,641,870). Indeed, the specification of the Zapata II reference contains additional text compared to the specification of application No. 08/425,763 and, thus, is more accurately a continuation-in-part. The portion of the Zapata II reference relied upon in the Office Action (column 14, lines 29-37 and column 15, lines 9-36) is, however, also present in application No. 08/425,763.

The Office Action acknowledges that the Zapata I reference does not teach a composition with a carrier or a fragment with an effector or reporter molecule and relies upon the Zapata II reference to cure these deficiencies. The Zapata II reference, however, does not cure the deficiencies of the Zapata I reference discussed above. In particular, the Zapata II reference does not teach or suggest that the Fab' fragment has a hinge region domain that comprises not more than one cysteine residue. Again, contrary to the assertions in the Office Action, the Zapata II reference reports that the Fab' fragment "contains a single free thiol." The Zapata II reference **does not** teach that the cysteine residue that forms a portion of the single free thiol is the only cysteine residue in a hinge region domain. In particular, the Office Action asserts that column 17, lines 35-43 of Zapata II teach a Fab' fragment that has been engineered to have one cysteine in a hinge region domain. Column 17 of

Zapata II discloses that a humanized anti-CD18 Fab' fragment was prepared having the light chain sequence SEQ ID NO:1 and heavy chain sequence SEQ ID NO:2. Lines 35- 43 appear to indicate that a free cysteine in the Fab' fragment was engineered to contain a free thiol in the hinge region. However, SEQ ID NO:2 in the Zapata II reference does not contain a complete hinge region domain, and further, the cysteines in SEQ ID NO:2 are not located in the hinge region domain. Since SEQ ID NO:2 in the Zapata II reference does not have a hinge region domain, Zapata II cannot therefore disclose attachment of a polymer to a single cystein residue in the hinge region domain as claimed in the present invention. Thus, the Zapata II reference does not cure the deficiencies of the Zapata I reference.

The claimed invention is, thus, not obvious in view of the combination of cited references. Accordingly, Applicants respectfully request that the rejection of claims 5 and 9-12 under 35 U.S.C. § 103(a) in view of the combination of the Zapata I and II references be withdrawn.

B. The Combination of the Jacobs and Bodmer References

Claims 5 and 9-12 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 5,853,723 (hereinafter, the "Jacobs reference") in view of WO 89/01974 (hereinafter, the "Bodmer reference"). The Jacobs reference is prior art under 35 U.S.C. § 102(a). The Office Action mistakenly asserts that it would have been *prima facie* obvious for one skilled in the art to have reduced the number of cysteine residues in the hinge region to one, as reported in the Bodmer reference and produce a Fab'-PEG antigen binding fragment reported by the Jacobs reference. Applicants traverse the rejection and respectfully request reconsideration for the reasons set forth below.

The Jacobs reference reports an anti-glutamic acid decarboxylase (GAD) Fab'-PEG molecule. The Jacobs reference reports that the PEG moiety provides a hydration shell around the monoclonal antibody fragment for inhibiting immune recognition thereof (see, column 6, lines 1-10). The Jacobs reference does not teach or suggest that the hinge region of the antibody fragment comprises not more than one cysteine residue.

The Bodmer reference reports altered antibody molecules having a hinge region which has a different number of cysteine residues from that found in the hinge region normally associated with the CH1 domain of the antibody molecule.

The Office Action provides three reasons that would allegedly have motivated one skilled in the art to modify the anti-GAD Fab'-PEG molecule of the Jacobs reference by reducing the number of cysteine residues to one. First, the Office Action asserts that the Jacobs reference teaches that the polymer provides a hydration shell around the monoclonal antibody fragment for inhibiting immune recognition (see, pages 6 and 7 of the Office action). One skilled in the art, however, would immediately recognize that this benefit that the polymer provides is independent of the number of cysteine residues present in the hinge region. Indeed, the hydration shell would be present whether there is one cysteine residue or ten cysteine residues.

The Office Action also asserts that one skilled in the art would have been motivated to modify the anti-GAD Fab'-PEG molecule of the Jacobs reference by reducing the number of cysteine residues to one for the purpose of "attaching other molecules (see page 7)" (see, page 6 of the Office Action). The portion of the Bodmer reference referred to in the Office Action (*i.e.*, page 7 of the Bodmer reference) reports one advantage of reducing the number of cysteine residues in the hinge region to 1. The advantage is that it will facilitate assembly of the antibody molecules because it will only be necessary to form a single disulphide bond. This advantage, however, is not applicable to Applicants' polymer modified **monovalent** antibody **fragments** because there is no assembly of antibody molecules called for in the claims. Further, the portion of the Jacobs reference referred to in the Office Action reports that the reduction to one cysteine residue will provide a specific target for attaching the hinge region to an effector or reporter molecule. In Applicants' claimed inventions, however, the polymer is linked to the cysteine residue. Thus, the cysteine residue would not be available for attachment of an effector or reporter molecule, in contrast to the linkage of a polymer molecule. Thus, the portion of the Bodmer reference referred to in the Office Action (*i.e.*, page 7 of the Bodmer reference) provides no motivation to combine the cited references in the manner suggested in the Office Action.

The Office Action also asserts that one skilled in the art would have been motivated to modify the anti-GAD Fab'-PEG molecule of the Jacobs reference by reducing the number of cysteine residues to one because it "reduces the complexity of subsequent chemical additions at the hinge (see page 10, Example 1)" (see, page 6 of the Office Action). The portion of the Bodmer reference referred to in the Office Action (*i.e.*, page 10, Example 1 of the Bodmer reference) reports a subsequent chemical addition to the hinge in which another Fab' molecule is crosslinked to form a $F(ab')_2$ molecule, which, as described above, is not Applicants' claimed invention. Thus, the portion of the Bodmer reference referred to in the Office Action (*i.e.*, page 10, Example 1 of the Bodmer reference) provides no motivation to combine the cited references in the manner suggested in the Office Action.

Thus, the claimed invention is not obvious in view of the combination of cited references. Accordingly, Applicants respectfully request that the rejection of claims 5 and 9-12 under 35 U.S.C. § 103(a) in view of the combination of the Jacobs and Bodmer references be withdrawn.

III. The Claims Are Clear And Definite

Claims 9-12 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants have amended the claims to be even more clear and definite. No new matter has been added. The scope of the claims has not been narrowed.

Claim 12 has been amended to delete the second occurrence of "poly(ethylene glycol)." Claim 12 has also been amended to delete recitation of "antigen binding" in the preamble. In addition, claim 12 has been amended to recite "hinge region domain" throughout the claim so that it is consistent with the phrase used in the specification (see, for example, page 6, lines 5-7 of the published PCT specification).

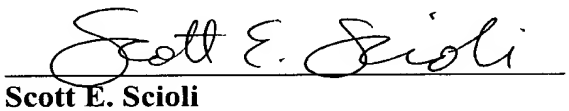
The Office Action mistakenly asserts that recitation of "said polymer" in claim 12 lacks antecedent basis. Claim 12, however, recites "A polymer modified monovalent antibody fragment..." Thus, recitation of "polymer" in the preamble provides sufficient antecedent basis for recitation of "said polymer" later in the claim. Thus, claim 12 is clear and definite.

In view of the foregoing, claims 9-12 are definite within the meaning of § 112. Accordingly, Applicants respectfully request that the rejection of claims 9-12 under 35 U.S.C. § 112, second paragraph be withdrawn.

IV. Conclusion

Applicants respectfully submit that the claims are in condition for allowance. An early notice of the same is earnestly solicited. The Examiner is invited to contact Applicants' undersigned representative at (215) 557-5986 if there are any questions regarding Applicants' claimed invention. Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

New claims 13-19 have been added.

Claims 9 and 12 have been amended as follows:

9. (Twice Amended) An antibody fragment according to claim 12 covalently attached to one or more [effector or] reporter molecules.

12. (Amended) A polymer modified monovalent antibody fragment, wherein said [antigen-binding] fragment comprises a heavy chain and a light chain, wherein

said heavy chain comprises [of] a VH domain covalently linked at its C-terminus to a CH1 domain extended to provide a hinge region domain, said hinge region domain comprising not more than one cysteine residue;

said light chain comprising [of] a VL domain, which is complementary to the VH domain, covalently linked at its C-terminus to a CL domain;

where not more than one polymer molecule is covalently attached to [said] the monovalent antibody fragment, said polymer molecule is linked to said cysteine residue in said hinge region domain of said heavy chain;

and wherein said polymer is an optionally substituted, straight or branched chain polymer selected from the group consisting of poly(ethylene glycol), [poly(ethylene glycol),] poly(propylene glycol), poly(vinyl alcohol) and derivatives thereof.

13. (New claim) An antibody fragment according to claim 12 covalently attached to one or more effector molecules.

14. (New claim) The fragment of claim 13 wherein the effector molecule is an antineoplastic agent, toxin, enzyme, nucleic acid, radionuclide, or chelated metal.

15. (New claim) The fragment of claim 9 wherein the reporter molecule is a chelated metal, fluorescent compound, or a compound detected by nuclear magnetic resonance spectroscopy or electron spin resonance spectroscopy.

16. (New claim) The fragment of claim 12 wherein the fragment binds a soluble antigen.

17. (New claim) The fragment of claim 19 wherein the soluble antigen is an interleukin, viral antigen, immunoglobulin, interferon, tumor necrosis factor-α, tumor necrosis factor-β, a colony stimulating factor, or a platelet derived growth factor, or a receptor thereof.

18. (New claim) The fragment of claim 12 wherein the polymer has an average molecular weight range from 25,000 Da to 40,000 Da.

19. (New claim) The fragment of claim 12 wherein a variable region domain of the fragment comprises at least one complementary determining region from one antibody and the remainder of the variable region domain from a second antibody.

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Antibody Engineering

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Carl A. K. Borrebaeck
Editor

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CHAPTER 3

Engineering the Hinge Region to Optimize Complement-induced Cytolysis

Inger Sandlie, Terje E. Michaelsen

The classical complement pathway is a cascade system generating a variety of potent biologic molecules. The pathway is triggered by the interaction of the first complement protein complex, C1, with antigen-complexed IgG. C1 is composed of C1q, C1r, and C1s, and the C1q subunit interacts with the second domain of the heavy chain (C_H2) on IgG. The residues Glu 318, Lys 320, and Lys 322 on C_H2 are involved in the binding.[1] This core binding motif is conserved in the four human IgG subclasses, both the lytic and the nonlytic molecules. Therefore, further structural determinants must be involved in the lysis mechanism. C1q has a molecular weight of approximately 460,000 and has the appearance of a bunch of tulips.[2] The molecule is multivalent in its binding to IgG, and binding to monomeric IgG is weak (Ka 10^4M^{-1}). When several IgGs bind to multiple epitopes on an antigenic surface, the resulting aggregation of IgG molecules allows the binding of two or more tulip heads, leading to a tight binding (Ka 10^8M^{-1}) that is necessary for the activation process to proceed.

It is possible that an exact alignment of antibodies and C1q is required for full activation and also that some degree of flexibility in the molecules is

Table 3-1. Comparison of Hinge Sequences of Human and Mouse Immunoglobulins

	Upper Hinge 216	*Middle Hinge*	*Lower Hinge 238*
Human IgG_1	EPKSCDKTHT	CPPCP	APELLGGP
Human IgG_2	ERK	CCVECPPCP	APPVAGP
Human IgG_3	ELKTPLGDTTHT	CPRCP (EPKSCDTPPPCPRCP)$_3$	APELLGGP
Human IgG_3M15	EPKS	CDTPPPCPRCP	APELLGGP
Human IgG_4	ESKYGPP	CPSCP	APEFLGGP
Mouse IgG_1	VPRDCG	CKPCICT	VPSEVS
Mouse IgG_{2a}	EPRGPTIKP	CPPCKCP	APNLLGGP

To study the proposed association between hinge length, flexibility, and effector function, mutants of human IgG_3 were made, varying the length of the hinge, while the rest of the molecule was kept unaltered.[12] Thus, the effect of manipulating the hinge could be studied independently of any effects due to isotype differences in C_H1, C_H2, and C_H3. The new antibodies were tested for their ability to activate complement and to initiate complement-mediated lysis (CML) and antibody-dependent cell-mediated cytotoxicity (ADCC). Five different deletion mutants were constructed, as shown in Figure 3-1, deleting one or more hinge exons. The resulting antibodies have hinges of 47, 45, 32, 15, and 0 amino acids, respectively. The length of the upper hinges of mutant M47 and M32 are identical to that of IgG_3 wild type, namely 12 amino acids, whereas the upper hinge of M45 and M15 consists of 4 amino acids. Both M45 and M15 activated complement. M45 was equally efficient

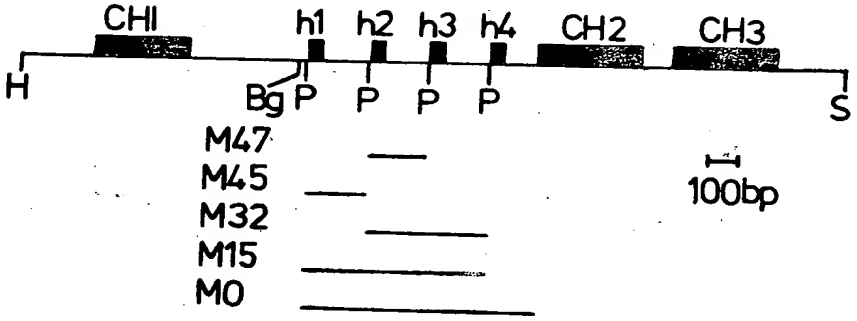

Figure 3-1. Restriction map of the human γ_3 gene. Exons are shown as boxes. Lines indicate the size of deletions in the hinge region in the various mutants. H = Hind III, Bg = Bg1 II, P = Pst I, S = Sph I.